

--The variant of the invention may further comprise a peptide extension at the N-terminal, e.g. consisting of 1-15 (particularly 4-10) amino acid residues, and specifically comprising 1, 2 or 3 positively charged amino acids. Some specific N-terminal peptide extensions are AS, SPIRR (SEQ ID. NO. 21), E1RP, E1SPIRPRP (SEQ ID. NO. 22), E1SPPRRP (SEQ ID. NO. 23) and E1SPIRPRP (SEQ ID NO:22). Further, any peptide extension described in WO 97/04079 and WO 97/07202 may be used.--

Please replace the paragraph on page 11, lines 21-27, with:

--Specific variants of the *H. lanuginosa* lipase are disclosed in the examples. Corresponding alterations may be made in other parent lipolytic enzymes. Further variants may be derived from these by omitting amino acid modifications at positions 1, 106, 186, 225, 232, 237, 239 or 274. Variants with 274S may optionally have a further C-terminal extension of WRRYRSAESVDKTRATMTDAELEKKLNSYVQM DKEYVKNNQARS (SEQ ID. NO. 24) (corresponding to the C-terminal of the *F. oxysporum* lipase) in full or truncated form.--

Please replace the paragraph on page 12, lines 1 – 6, with:

T267stop indicates a stop codon, i.e. deletion of T267 and all following amino acids (i.e. C268 and L269). 270P, 271V indicates a C-terminal extension of PV (i.e. at new positions 270 and 271). -G266 indicates deletion of G at position 266. Parentheses indicate that the alteration is optional, or in examples that the alteration is uncertain. SPIRR (SEQ ID NO:21) indicates an N-terminal extension. D266 may refer to the position or to substitution with any amino acid (except D).

Please replace the paragraph on page 12, lines 7-10, with:

--E1SPPCGRRP (SEQ ID. NO. 25) or SPPCGRRP(-E) (SEQ ID NO.25) indicates a substitution of E1 with SPPCGRRP (SEQ ID NO:25), i.e. a peptide addition at the N-terminal. T267GS indicates a substitution of T267 with GS, or in other words the substitution T267G and an insertion of S between G267 and C268.--

Please replace the paragraph on page 33, lines 15-26, with:

--The gene encoding the lipolytic enzyme in question is inserted into the plasmid pHD414. In accordance with the manufacturer's instructions the *ScaI* site of the Ampicillin gene of pHD414 is changed to a *MluI* site by use of the following primer:

Primer 3: AGAAATCGGGTATCCTTTCAG. (SEQ I.D. NO. 27)

The pHD414 vector comprising the lipolytic gene in question is then used as a template for DNA polymerase and oligos 7258 and 7770.

7258: 5'p gaa tga ctt ggt tga cgc gtc acc agt cac 3' (SEQ I.D. NO. 28)

(Thus changing the *ScaI* site found in the ampicillin resistance gene and used for cutting to a *MluI* site).

Primer no. 7770 was used as the selection primer.

7770: 5'p tct agc cca gaa tac tgg atc aaa tc 3' (SEQ I.D. NO 29) (Changes the *ScaI* site found in the *H. lanuginosa* lipase gene without changing the amino acid sequence).

Please replace the paragraph on page 37, lines 1-4, with:

SPIRR (SEQ ID NO:21), G91A, D96W, E99K, W260C, G263Q, L264A, I265T, G266D, T267A, L269N, 270A, 271G, 272, G273F, (274S)

SPIRR (SEQ ID NO:21), G91A, D96W, E99K, G263Q, L264A, I265T, G266D, T267A, L269N, 270A, 271G, 272G, 273F, (274S)

Please replace the paragraph on page 38, lines 7-10, with:

SPIRR (SEQ ID NO:21), D96W, E99K, G263Q, L264A, I265T, G266D, T267A, L269N, 270A, 271G, 272G, 273F, (274S)

SPIRR (SEQ ID NO:21), G91A, D96W, G263Q, L264A, I265T, G266D, T267A, L269N, 270A, 271G, 272G, 273F, (274S)

Please replace the table under Example 5, beginning on page 41, with:

E1A, G91A, D96W, E99K, P256A, W260H, G263Q, L264A, I265T, G266D, T267A, L269N, 270A, 271G, 272G, 273F, (274S)
SPIRR (SEQ ID NO:21), G91A, D96W, E99K, G263Q, L264A, I265T, G266D, T267A, L269N, 270A, 271G, 272G, 273F, (274S)
E1A, G91A, D96W, P256A, W260H, G263Q, L264A, I265T, G266D, T267A, L269N, 270A, 271G, 272G, 273F, (274S)

E1A, G91A, D96W, E99K, P256A, W260H, G263Q, L264A, I265T, G266D, T267A, L269N
E1A, G91A, D96W, E99K, Q249R, G266S, 270D, 271G
E1A, G91A, D96W, E99K, Q249R, G266D
E1A, G91A, D96W, E99K, Q249R, G266A, 270P, 271G
G266D
E1SPPCGRRP (SEQ ID NO:25) +E99N +E239C +Q249R +G266D
E1SPPCGRRP (SEQ ID NO:25) +E239C +Q249R +G266D
E1SPPCGRRP (SEQ ID NO:25) +L93K +E99K +E239C +Q249R +G266D
E1SPPCGRRP (SEQ ID NO:25) +E99K +E239C +Q249R +G266D
G266A
G266W
G266V
G263Q +L264A +I265T +G266D +T267A
G263F +L264A +G266S +T267E
E1SPPCGRRP (SEQ ID NO:25) +E239C +Q249R +G263Q +L264A +I265T +G266D +T267A
G266S
G266L
G263A +G266A
G263A +G266Y
E1SPPCGRRP (SEQ ID NO:25) +E239C +Q249R +G266A
E1SPPCGRRP (SEQ ID NO:25) +E239C +Q249R +G266S
E1SPPCGRRP (SEQ ID NO:25) +E239C +Q249R +G263F +L264A +G266S +T267E
D62A + G266A
D62A + G266S
D96S + G266A
D96S+ G266S
D96S+ G266R
D96S+ G266W
D96S+ G266V
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ G266D
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ G266S
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ G263E+ G266S+ 270A

E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ L264P+ G266S
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ P256T+ G266D
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ G266C+ T267P+ L269stop
G263D +L264I +I265N +G266E +T267GS
E219G +L264I +I265N +G266T +T267GL
E1A+ G91A+ D96W+ E99K+ P256A+ W260H+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G+ 273F (+274S)
E1A+ G91A+ D96W+ E99K+ E239C+ Q249R+ P256A+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G +273F (+274S)
E1A+ G91A+ D96W+ E99K+ N248T+ Q249R+ W260Q+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G+ 273F (+274S)
SPIRR (SEQ ID NO:21) + G91A+ D96W+ E99K+ W260C+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272+ G273F (+274S)
SPIRR (SEQ ID NO:21) + G91A+ D96W+ E99K+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G+ 273F (+274S)
E1A+ G91A+ D96W+ E99K+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G+ 273F (+274S)
E1A+ G91A+ D96W+ E99K+ P256A+ W260H+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G +272G +273F (+274S)
SPIRR (SEQ ID NO:21) + D96W+ E99K+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G+ 273F (+274S)
SPIRR (SEQ ID NO:21) + G91A+ D96W+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G+ 273F (+274S)
E1A+ G91A+ D96W+ E99K+ P256A+ W260H+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N
E1A+ G91A+ D96W+ E99K+ Q249R+ G263E+ G266D+ L269N+ 270P+ 271V+ 272G+ 273F
E1A+ G91A+ D96W+ E99K+ Q249R+ G263A+ G266S+ L269N+ 270A+ 271G+ 272R+ 273F
E1A+ G91A+ D96W+ E99K+ Q249R+ L264P+ Δ266+ L269I+ 270P+ 271R+ 272G+ 273F
E1A+ G91A+ D96W+ E99K+ Q249R+ L264C+ I265N+ G266P+ T267stop
E1A+ G91A+ D96W+ E99K (+R232L)+ Q249R+ G266S+ 270A
E1A+ G91A+ D96W+ E99K+ Q249R+ G266S+ 270D+ 271G
E1A+ G91A+ D96W+ E99K+ Q249R+ L264F+ Δ266+ 270A+ 271G+ 272G+ 273F
E1A+ G91A+ D96W+ E99K+ Q249R+ L264G+ I265G+ G266F+ T267stop
E1A+ G91A+ D96W+ E99K+ Q249R+ L264stop
E1A+ G91A+ D96W+ E99K+ P256A+ W260H+ G263Q+ L264A+ I265T+ G266D+ T267A+

L269N+ 270A+ 271G
E1A+ G91A+ D96W+ E99K+ P256A+ W260H+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G
E1A+ G91A+ D96W+ E99K+ Q249R+ G266D
E1A+ G91A+ D96W+ E99K+ Q249R+ G266D
E1A+ G91A+ D96W+ E99K+ Q249R+ G266A+ 270P+ 271G
E1A+ G91A+ D96W+ E99K+ Q249R+ L264P+ I265F+ L269stop
E1A+ G91A+ D96W+ E99K+ Q249R+ G266D+ L269S+ 270A+ 271G+ 272G+ 273F
E1A+ G91A+ D96W+ E99K+ Q249R+ G266D+ L269N+ 270A
E1A+ G91A+ D96W+ E99K+ Q249R+ G266S+ L269N+ 270A+ 271G+ 272G+ 273F
E1A+ G91A+ D96W+ E99K+ Q249R+ L264P+ L267Q+ L269N
E1A+ G91A+ D96W+ E99K+ Q249R+ G263R+ I265L+ L269N+ 270P
E1A+ D96W+ E99K+ P256A+ W260H+ G263Q+ L264A+ I265T+ G266D+ T267A+ L269N+ 270A+ 271G+ 272G+ 273F (+274S)
E1A+ G225R+ G266D
E1A+ G225R+ G263A+ I265V+ G266S
E1A+ G225R+ G263A+ T267A
E1SPPCGRRP (SEQ ID NO:25) + D96S+ E239C+ Q249R+ I252M+ L264Q+ G266D
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ G266D
E1SPPCGRRP (SEQ ID NO:25) + D96S+ E239C+ Q249R+ G266D
E1SPPCGRRP (SEQ ID NO:25) + D96S+ E239C+ Q249R+ G266C+ L267A
E1A+ G91A+ D96W+ E99K+ Q249R+ G266A
E1A+ D96M+ G106S+ G225R+ G266D
E1A+ D96Q+ G106S+ G225R+ G266S
E1A+ D96F+ G225R+ G266S
E1A+ D96C+ G225R+ G266T
E1A+ D96H+ G106S+ G225R+ G266S
SPIRR (SEQ ID NO:21) + D96S+ G266D
SPIRR (SEQ ID NO:21) + D96R+ G106S+ G266D
SPIRR (SEQ ID NO:21) + D96I+ G106S+ G266S
SPIRR (SEQ ID NO:21) + D96W+ K237R+ G266S
SPIRR (SEQ ID NO:21) + G266A
SPIRR (SEQ ID NO:21) + D96S+ G106S+ G225R+ G266D

SPIRR (SEQ ID NO:21) + D96Q+ G106S+ G225R+ G266A
SPIRR (SEQ ID NO:21) + D96Y+ G106S+ G225R+ G266N
SPIRR (SEQ ID NO:21) + D96C+ G106S+ G225R+ G266T
SPIRR (SEQ ID NO:21) + D96H+ T186I+ G225R+ G266S
E1SPRRRP (SEQ ID NO:23) + G91A+ D96W+ E239C+ Q249R+ G266D
E1SPRRRP (SEQ ID NO:23) + G91A+ D96W+ E239C+ Q249R+ G266S
E1SPRRRP (SEQ ID NO:23) + G91A+ D96W+ E239C+ Q249R+ G263E+ G266S+ 270A
E1SPRRRP (SEQ ID NO:23) + G91A+ D96W+ E239C+ Q249R+ L264P+ G266S
E1SPRRRP (SEQ ID NO:23) + G91A+ D96W+ E239C+ Q249R+ P256T+ G266D
E1SPRRRP (SEQ ID NO:23) + G91A+ D96W+ E239C+ Q249R+ G266C+ T267P+ L269stop
E1A+ G91A+ D96W+ E99K+ Q249R+ G266S+ T267S
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ P256T+ G266S
E1SPPCGRRP (SEQ ID NO:25) + E239C+ Q249R+ P256T+ G266S+ T267A
E1SPPCGRRP (SEQ ID NO:25) + E239C+ Q249R+ G266D
E1SPPCGRRP (SEQ ID NO:25) + G91A+ D96W+ E239C+ Q249R+ G266D
E1SPRRRP (SEQ ID NO:23) + D96S+ E239C+ Q249R+ G266D
L259S
G266D
G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A+ 271G+ 272G+ 273F (+274S)
G266E
G263A +G266A
E1SPCRPRP (SEQ ID NO:26) +E239C +Q249R +G266A
E1SPCRPRP (SEQ ID NO:26) +E239C +Q249R +G266S
D96S + G266A
D96S + G266S
D96S + G266W
E1SPPCGRRP (SEQ ID NO:23) +D96S +E239C +Q249R +G263D +L264I +I265N +G266E +T267GS
E1SPPCGRRP (SEQ ID NO:23) +D96S +E239C +Q249R +L264I +I265N +G266T +T267GL
D96F +G266A
D96F +G266S
E1SPPCGRRP (SEQ ID NO:23) +E99N +E239C +Q249R +G266A

E1SPPCGRRP (SEQ ID NO:23) + D96S +E239C +Q249R +G266A
E1SPPCGRRP (SEQ ID NO:23) + D96S +E239C +Q249R +G266S
E1SPPCGRRP (SEQ ID NO:23) + D96S +E239C +Q249R +G263F +L264A +G266S +T267E
V60G +D62A +S83T +R84K +D96W +G266D
V60G +D62A +S83T +D96W +G266D
V60G +D62A +S83T +D96W +G266W
L259I
L259N
D96W +G263Q +L264A +I265T +G266D +T267A

Please replace the table beginning on page 49, below Example 7, with:

E1SPIRPRP (SEQ ID NO:22) +G91A +D96N +E99K +Q249R
E1SPCRPRP (SEQ ID NO:26) + S83T+ N94K+ D96L+ E239C+ Q249R
G266D
E1SPIRPRP (SEQ ID NO:22) +D62A +E99K +Q249R
E1SPIRPRP (SEQ ID NO:22) +D62G +E99K +Q249R
E1SPIRPRP (SEQ ID NO:22) +D62V +E99K +Q249R
E1SPIRPRP (SEQ ID NO:22) +R84W +E99K +Q249R
E1SPIRPRP (SEQ ID NO:22) +R84K +E99K +Q249R
E1SPIRPRP (SEQ ID NO:22) + K98D +E99K +Q249R
E1SPIRPRP (SEQ ID NO:22) + E99K +Q249R + 270PGLPFKRV
E1SPPCGRRP (SEQ ID NO:25) + E99N +N101S +T231K +R232G +D234G +E239C +Q249R
E1SPIRPRP (SEQ ID NO:22) +E99K +Q249R + 270PWPARLGRL
L93K +D96G
G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A+ 271G+ 272G+ 273F (+274S)
E1SPCRPRP +V60G +E99N +S119G +R209P +E239C +Q249R
G266A
G266E
G266V
G263Q +L264A +I265T +G266D +T267A
G266L

G263A +G266A
E1SPCRPRP (SEQ ID NO:26) +E239C +Q249R +G266A
E1SPCRPRP (SEQ ID NO:26) +E239C +Q249R +G266S
D96S + G266A
D96S + G266S
D96S + G266W
L264I +I265N +G266T +T267GL
E1SPPCGRRP (SEQ ID NO:25) +D96S +E239C +Q249R +L264I +I265N +G266T +T267GL
D96F +G266A
D96F +G266S
E1SPPCGRRP (SEQ ID NO:25) +E99N +E239C +Q249R +G266A
E1SPPCGRRP (SEQ ID NO:25) + D96S +E239C +Q249R +G266A
E1SPPCGRRP (SEQ ID NO:25) + D96S +E239C +Q249R +G266S
D62A + S83T
E1SPPCGRRP (SEQ ID NO:25) +K98D +E99N +E239C +Q249R
T231R +N233R +270CP
E1SPPCGRRP (SEQ ID NO:25) +E99N +E239C +Q249R +270MD
E1SPPCGRRP (SEQ ID NO:25) + D62A +S83T +E99N +E239C +Q249R
D62A +S83T + G91A +E99K +T231R +N233R +Q249R
V60G +D62A +S83T +R84K +D96W +G266D
L259N
L259R
L259M
L259Q
SPPCGRRP(-E) (SEQ ID NO:25) +R84W +E99N +N101S +E239C +Q249R
R84W +G91A +E99K +T231R +N233R +Q249R
Y21I
Y21V
SPIRPRP(-E) (SEQ ID NO:22) +R84L +E99K +Q249R
Y21C
SPIRPRP(-E) (SEQ ID NO:22) +D62 +E99K +Q249R
D96W +G263Q +L264A +I265T +G266D +T267A +L269N +A270 +G271 +G272 +F273 +S274.

G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S

The following variants of the parent lipase from *Humicola lanuginosa* may also have an increased specificity for long-chain fatty acids:

SPIRPRP(-E) (SEQ ID NO:22) +V60R +D62V +L93K +E99K +Q249R
SPIRPRP(-E) (SEQ ID NO:22) +D62V +E99K +Q249R
SPIRPRP(-E) (SEQ ID NO:22) +E99K +Q249R +P256D
SPIRPRP(-E) (SEQ ID NO:22) +D62V +E99K +Q249R +P256D
SPIRPRP(-E) (SEQ ID NO:22) +D62V +E99K +Q249R +P256S
G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96L +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96N +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96A +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96E +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96S +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96R +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96G +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96Q +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96F +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96W +E99K +G263Q +L264A +I265T +G266S +T267A +L269N +270A +271G +272G +273F +274S
G91A +D96F +E99K +G263Q +L264A +I265T +G266S +T267A +L269N +270A +271G +272G +273F +274S
R84W +G91A +D96W +E99K +G263Q +L264A +I265T +G266S +T267A +L269N +270A +271G +272G +273F +274S
R84W +G91A +D96F +E99K +G263Q +L264A +I265T +G266S +T267A +L269N +270A +271G +272G +273F +274S
R84W +G91A +D96F +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270A

